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Effect of 11CFT and 11C33 inoculants on the chemical and fermentation composition, and aerobic stability of corn silage during the feed out period

Efeito dos inoculantes 11CFT e 11C33 sobre a composição químico-fermentativa e estabilidade aeróbia da silagem de milho durante o período de utilização da silagem

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Highlights

Bacterial inoculants improved the *in situ* digestibility of corn silage.
Bacterial inoculants caused no variations in temperature and pH at silage unloading.
L. buchneri, L. plantarum and *E. faecium* provided better aerobic stability.

Abstract

The objective was to evaluate the efficiency of two bacterial inoculants, 11CFT and 11C33, with different genera of lactic acid bacteria on the chemical and fermentation composition of the silage, and the temperature and pH behavior of the silage during the feed out period. The experimental design used was randomized blocks, with three treatments: corn silage without inoculant (control); corn silage with 11CFT inoculant (consisting of strains of *Lactobacillus buchneri* and *L. casei*); and corn silage with 11C33 inoculant (consisting of strains of *L. buchneri*, *L. plantarum* and *Enterococcus faecium*). The use of both inoculants increased the concentration of lactic acid in the silage (22.42 g kg^{-1} for control against 36.00 and 33.33 g kg^{-1} for 11CFT and 11C33, respectively) and reduced aerobic dry matter losses. The silage treated with 11C33 obtained a higher concentration of acetic acid (17.44 g kg^{-1}) and propionic acid (2.08 g kg^{-1}). The 11CFT

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inoculant provided a lower concentration of ethanol, however, without differing from the silage with 11C33 (0.70 and 1.61 g kg^{-1}, respectively). Even without variations in temperature and pH at silage unloading, the use of the 11C33 inoculant generated a higher concentration of acetic and propionic acid, providing better aerobic stability days after unloading. Both inoculants also improved the *in situ* ruminal digestibility of corn silage compared to control silage. They provide an increase in the content of lactic and propionic acids, which assist to reduce dry matter losses and ethanol production. There were no variations in temperature and pH at the silo unloading, however, the use of the 11C33 inoculant generated a higher concentration of acetic and propionic acids providing better aerobic stability after exposure to air.
Key words: Organic acids. Lactic acid bacteria. *Lactobacillus buchneri*. *L. plantarum*. Dry matter losses.

Resumo

Objetivou-se avaliar a eficiência de dois inoculantes bacterianos, 11CFT e 11C33, com diferentes gêneros de bactérias ácido láticas sobre a composição químico-fermentativa da silagem, bem como o comportamento da temperatura e pH da silagem durante sua utilização para alimentação. O delineamento experimental utilizado foi o de blocos casualizados, composto por três tratamentos: silagem de milho sem inoculante (controle); silagem de milho com inoculante 11CFT (constituído por cepas de *Lactobacillus buchneri* e *L. casei*); e silagem de milho com inoculante 11C33 (constituído por cepas de *L. buchneri*, *L. plantarum* e *Enterococcus faecium*). A utilização de ambos inoculantes aumentou a concentração de ácido lático da silagem (22,42 g kg^{-1} para controle contra 36,00 e 33,33 g kg^{-1} para 11CFT e 11C33, respectivamente) e reduziram as perdas de matéria seca em aerobiose. A silagem tratada com 11C33 obteve maior concentração de ácido acético (17,44 g kg^{-1}) e propiônico (2,08 g kg^{-1}). O inoculante 11CFT proporcionou menor concentração de etanol, porém, sem diferir da silagem com 11C33 (0,70 e 1,61 g kg^{-1}, respectivamente). Mesmo sem haver variações de temperatura e pH no momento da desensilagem, o uso do inoculante 11C33 gerou maior concentração de ácido acético e propiônico proporcionando melhor estabilidade aeróbia após a desensilagem. Ambos inoculantes também melhoraram a digestibilidade *in situ* da silagem de milho em comparação com a silagem controle. Eles proporcionam aumento no teor dos ácidos lático e propiônico, que auxiliaram na redução das perdas de matéria seca e produção de etanol. Não ocorreram variações de temperatura e pH logo após abertura do silo, porém, o uso do inoculante 11C33 gerou uma maior concentração de ácidos acético e propiônico proporcionando melhor estabilidade aeróbia após a exposição ao ar.
Palavras-chave: *Ácidos* orgânicos. Bactéria *ácido* lática. *Lactobacillus buchneri*. *L. plantarum*. Perdas de matéria seca.

Introduction

The principle of silage fermentation involves the production of organic acids, primarily acetic and lactic acid, through the metabolization of soluble carbohydrates by lactic acid bacteria in an anaerobic environment (Jobim & Nussio, 2013). At first, there is the production of acetic acid by heterofermentative bacteria, which has a primary function in controlling molds and yeasts after opening the silo and exposing the silage to air. This group of bacteria can be subdivided into obligate and facultative (Holzer, Mayrhuber, Danner, & Braun, 2003), the former uses hexoses for the production of lactic and/

or acetic acid, and pentoses for the production of acetic acid, exclusively. Facultative bacteria can produce both acids from hexoses and pentoses, depending on the final electron receptor.

Namely, lactic acid is considered a strong acid responsible for stabilizing the pH of the ensiled forage and controlling spoilage bacteria (Tabacco, Piano, Revello-Chion, & Borreani, 2011). However, the faster the stabilization of this pH, the lower the control of fungi and yeasts by acetic acid, which can culminate in a silage with low aerobic stability, which is the main reason why the use of homofermentative bacteria strains is restricted in inoculants (Lynch, Baah, & Beauchemin, 2015).

The production of commercial bacterial inoculants comprises mostly blends of species of lactic acid bacteria, in which *Lactobacillus plantarum* is the most commonly used, and often *Lactobacillus casei*, *Lactobacillus buchneri*, in addition to other species such as *Pediococcus faecium* and *Enterococcus faecium* (Muck, 2010). The main function of these inoculants is to promote higher concentrations of organic acids during fermentation (M. S. J. Silva, Jobim, Poppi, Tres, & Osmari, 2015); however, it is important to consider that inoculants with different combinations of bacteria have different functions and provide different results (M. R. Oliveira, Neumann, Oliboni, Gobetti, & Faria, 2011). In a meta-analysis, A. S. Oliveira et al. (2017) concluded that the use of inoculants with lactic acid bacteria can improve the chemical and fermentation characteristics, decrease losses of dry matter and nutrients in corn silages. However, the authors warn that different combinations of bacteria provide different results.

In this context, the goal of this study was to evaluate the efficiency of two bacterial inoculants with different genera of lactic acid bacteria on the chemical and fermentation composition of the silage, as well as the temperature and pH behavior of the silage during the feed out period.

Material and Methods

The experiment was conducted in the Laboratory of Food Analysis and Nutrition of Ruminants and in the Didactic unit for research and extension in Beef Cattle - Confinement of the Animal Production Center (NUPRAN) along with the Master's degree Program in Veterinary Sciences of the Agricultural and Environmental Sciences Sector, State University of the Midwest (CEDETEG/UNICENTRO), located in Guarapuava, State of Paraná, Brazil (25º23'02 "S and 51º29'43" W).

For making the silage, the corn hybrid P 2866 H was harvested at the hard-grain stage and chopped with a Pecus 9004 machine (Nogueira®), set to 15 mm sting size. The inoculants were diluted in water, according to the manufacturer's instructions, to obtain a concentration of 20 × 1010 CFU g^{-1} of the product per ton of fresh biomass, using a variable pressure sprayer. The application was made during the corn harvest, using a spray nozzle located in the unloading chute of the forage machine, seeking a uniform distribution of the product.

The experimental design used was randomized blocks, with three treatments: T_1: corn silage without inoculant (control); T_2: corn silage with Pioneer® 11CFT inoculant (strains of *Lactobacillus buchneri* [1.1 × 10^{11} CFU g^{-1}] and

Lactobacillus casei [1.1 × 10^{11} CFU g^{-1}]); and T_3: corn silage with Pioneer® 11C33 inoculant (strains of *Lactobacillus buchneri* [1.1 × 10^{11} CFU g^{-1}], *Lactobacillus plantarum* [1.1 × 10^{11} CFU g^{-1}] and *Enterococcus faecium* [1 × 10^{10} CFU g^{-1}]), and four repetitions each, where each repetition was represented by a storage silo.

Experiment 1. Dry matter losses, chemical and fermentation composition and in situ digestibility

The forage collected from each treatment was stored in trench silos 15 m long, 4 m wide and 1.2 m high, sealed with silage tarp double side of polyethylene (150 µm). The opening of the silos occurred simultaneously at 160 days after sealing. During the ensiling, eight bags of malleable nylon 100% polyamine, with 85 µm pores and 12 x 50 cm in size, containing silage were randomly allocated inside each silo. Each bag was identified, weighed individually empty, and weighed again after filling. Nylon clamps were used to seal the bags. After the removal of each bag, it was weighed and dry matter was measured, and this procedure allowed to estimate the DM losses from silage.

Daily, at 07h30 and 16h30, temperature was measured in silages of the different treatments at three points at the top layer and three points at the bottom layer of each silo using a metal rod digital thermometer, as well as the ambient temperature. The temperature measurements in the silage, in the referred strata, were carried out at a depth of 7 cm in the structured mass at the silo face. In the same period, pH was read by removing the silage for use in animal feed, through a digital potentiometer.

Silo unloading was divided in four periods of evaluation, three periods of 28 days and the fourth with 21 days. The extraction of silage from each silo to feed the animals occurred with a daily advance of 15 cm. Homogeneous silage samples were collected in these different evaluation periods, weighed and pre-dried in an air forced oven at 55 ºC to constant weight, for analysis of partial dry matter (DM) content, being sequentially ground in a Willey mill, with a 1 mm mesh sieve. The pre-dried samples were analyzed for total dry matter in an oven at 105 ºC for 4 hours, crude protein (CP) by the micro Kjeldahl method, and the mineral matter (MM) by incineration at 550 °C for 4 hours. The contents of neutral detergent fiber (NDF), acid detergent fiber (ADF) and lignin (LIG) were determined according to Silva and Queiroz (2009).

The dry matter digestibility of the materials was estimated by the *in situ* technique using nylon bags with 12 × 8 cm in size and 40 - 60 µm pores, containing 5 g dry sample of each material milled to 1 mm, for later incubation in the rumen. The incubation times used were 24 and 48 hours in two steers with 36 months of age, average body weight of 550 kg, with permanent ruminal fistula.

The concentration of lactic acid was determined according to the methodology described by Price (1969). The concentrations of ethanol, and acetic, propionic, butyric, valeric and isovaleric acids in the samples were determined by gas chromatography using a Shimadzu® GC-2010 Plus chromatograph equipped with an AOC-20i auto injector, Stabilwax-DA™ capillary column (30 m, 0.25 mm ID, 0.25µm df, Restek®) and flame ionization detector (FID), after acidification with 1 M phosphoric acid p.a. (Ref. 100573, Merck®) and

fortification with the WSFA-2 standard (Ref. 47056, Supelco®). The sample collected was 15 g and was homogenized in a blender with 200 mL distilled water. After homogenizing for one minute, the sample was sieved and centrifuged, a volume of 1 µL was taken. This sample was injected at a 40:1 split ratio, using helium as carrier gas at a flow rate of 42 cm s^{-1}, obtaining the separation of the analyses in a chromatographic run of 11.5 minutes. The injector and detector temperatures were 250 ºC and 300 ºC, respectively, and the initial column temperature was 40 ºC. The column temperature ramp started with a gradient from 40 to 120ºC at a rate of 40ºC min^{-1}, followed by a gradient from 120 to 180 ºC at a rate of 10 ºC min^{-1}, and from 180 to 240 ºC at a rate of 120 ºC min^{-1}, keeping the temperature at 240 ºC for another 3 minutes at the end. For the quantification of analytes, a calibration of the method was made with dilutions of the WSFA-2 standard (Ref. 47056, Supelco®), glacial acetic acid (Ref. 33209, Sigma-Aldrich©) and HPLC grade ethanol (Ref. 459828, Sigma-Aldrich®) analyzed under the conditions described above. The detection and integration of the peaks were made using the GCsolution 2.42.00 software (Shimadzu®).

Experiment 2. Aerobic stability

Aerobic stability assessments were performed twice during the silo unloading period. The first evaluation was carried out 40 days after opening the silos and the second, 83 days after opening the silo. The silage of each silo was loosened to facilitate the exposure of the ensiled material to air, and a 400 g sample of the material was placed in buckets with a capacity of 1 kg. The buckets were stored in a controlled environment, with a temperature set to remain stable at 25 °C, throughout the evaluation period. To determine aerobic stability, daily at 06h00, 14h00 and 22h00 hours, temperature and pH were read and contents of dry matter and mineral matter of the evaluated silages were determined. The evaluation time was maintained until the loss of aerobic stability of the material.

The silage temperature was measured with a digital long-stemmed thermometer model Gulterm 1001 inserted at the center of the forage mass. The pH readings were measured using a digital potentiometer. In a second set of buckets, samples were taken daily, weighed, and pre-dried in a forced-air oven at 50ºC to constant weight to determine the partial dry matter content, sequentially ground in a Willey mill, with a 1 mm mesh sieve. In the ground samples, the contents of dry matter (DM) and mineral matter (MM) were determined, according to Silva and Queiroz (2009). The criterion for defining aerobic stability break was considered when the pH increased in levels above 0.5 units in up to five days of evaluation, as mentioned by Weinberg et al. (2007). The aerobic stability of corn silages treated with different inoculants, at different times of silo unloading (40 and 83 days), was promoted by the evaluation of the final contents of dry matter and mineral matter, as well as by the time to achieve maximum temperature and increase of pH.

Statistical analysis

Data related to dry matter losses, the chemical and fermentation composition and *in situ* digestibility of DM, as well as the average contents of dry matter and mineral matter, and

pH in each evaluation periods of the different silages were subjected to the Shapiro-Wilk and Bartlett tests to check the assumptions of normality and homogeneity of variance, respectively. Once these assumptions were met, the F-test was applied at 5% probability of confidence, by analysis of variance (ANOVA) and then the Tukey's test to compare multiple means at 5% significance. The temperature and pH data were also subjected to polynomial regression analysis, considering the variable hours of evaluation, using the PROCREG procedure of SAS software (1993).

Results and Discussion

The use of inoculant was not able to cause differences regarding the contents of dry matter, mineral matter, crude protein, acid detergent fiber and lignin, with average values of 38.82%, 2.40%, 5.86%, 26.74% and 4.70%, respectively (Table 1), regardless the time the material remained ensiled. The fiber content in neutral detergent was higher for silage inoculated with 11C33 (46.72%), while the control and silage inoculated with 11CFT did not differ from each other (41.00 and 43.17%, respectively). The 24h-DMD of silage was not affected by the use of inoculants, with an average of 54.47%. However, the 48h-DMD of both treated silages was higher compared to the control silage. Fugita et al. (2012) and Abdel-Rahman, Tashiro and Sonomoto (2011) explained that the performance of some enzymes produced by lactic acid bacteria facilitate the use of fiber, proving that higher fiber content in food is not synonymous with less digestibility.

Both inoculants resulted in lower levels of dry matter loss in anaerobiosis, indicating that the greater and more accelerated production of lactic acid has reduced the concentration of spoilage aerobic microorganisms in the aerobic phase that precedes the stabilization after silo sealing. According to Borreani, Tabacco, Schmidt, Holmes and Muck (2018), facultative heterofermentative bacteria, such as *L. casei* and *L. plantarum,* are justified by the lactic acid-producing capacity and markedly lowering the pH of the ensiled mass.

Kleinschmit and Kung (2006) showed that the isolated use of *L. buchneri* increased dry matter losses. Likewise, Filya and Sucuc (2010) found greater losses of dry matter with the isolated use of *L. buchneri*, while the use of *L. plantarum* reduced the losses. The inoculants in this study are facultative and obligate heterofermentative bacteria combinations, leading to greater benefits (A. S. Oliveira et al., 2017). Rabelo et al. (2012) also evaluated the effects of combinations of inoculants with facultative heterofermentative bacteria in corn silage and found that the use of all contributed to the reduction of dry matter losses.

Table 1
Chemical composition, *in situ* ruminal digestibility and dry matter losses of corn silage treated with different bacterial inoculants

Parameter	Silage			Average	*P*-value	SEM
	Control	11CFT	11C33			
DM, %	39.00	40.11	39.45	38.82	0.9246	1.2528
MM, %	2.49 a	2.23 b	2.50 a	2.40	0.0354	0.0750
CP, %	5.82	5.57	6.21	5.86	0.1963	0.2385
aNDF, %	41.00 b	43.17 b	46.72 a	43.63	0.0073	1.5005
ADF, %	27.36	25.87	27.02	26.74	0.5749	1.0326
LIG, %	4.83	4.49	4.79	4.70	0.7329	0.3327
24h-DMD, %	56.67	54.71	52.05	54.47	0.3242	2.0951
48h-DMD, %	59.94 b	68.86 a	65.64 a	64.81	0.0002	1.1297
Dry matter losses, %	9.62 a	5.33 b	4.74 b	6.63	0.0463	2.0785

Averages, followed by different letters in the same row, differ by the Tukey Test at 5%.

Along with lower dry matter losses, the silages inoculated with 11CFT and 11C33 also showed a higher concentration of lactic acid in their composition (36.00 and 33.33 g kg^{-1}, respectively; Table 2). The high concentration of lactic acid stimulates the drop in pH accelerating the stability of the silage (Jobim & Nussio, 2013). However, after opening the silo, lactic acid becomes a readily available substrate for yeasts, enhancing their development and nutrient consumption, providing a silage with low aerobic stability (Wilkinson & Davies, 2013). These indicate the importance of an assertive combination of bacteria in the inoculant, since the production of acetic acid to control yeasts after opening is fundamental. The 11C33 inoculant produced a silage with a greater participation of acetic acid (17.44 g kg^{-1}) compared to the other treatments.

Table 2
Fermentation profile (g kg^{-1}) of corn silage treated with different bacterial inoculants

Parameter	Silage			Average	*P*-value	SEM
	Control	11CFT	11C33			
	g kg^{-1} de DM					
Latic Acid	22.42 b	36.00 a	33.33 a	30.58	0.0001	0.6560
Acetic Acid	11.49 b	9.63 b	17.44 a	12.85	0.0036	0.6712
Propionic Acid	0.22 c	1.22 b	2.08 a	1.18	0.0001	0.0686
Isobutyric Acid	0.00 b	0.24 a	0.06 b	0.10	0.0001	0.0097
Butyric Acid	0.06 b	0.27 a	0.14 ab	0.16	0.0237	0.2455
Isovaleric Acid	0.00 c	0.18 a	0.10 b	0.09	0.0001	0.0073
Valeric Acid	0.00 b	0.31 a	0.09 b	0.13	0.0023	0.0242
Ethanol	1.81 a	0.70 b	1.61 ab	1.37	0.0300	1.4376

Averages, followed by different letters in the same row, differ by the Tukey Test at 5%.

Ethanol is basically produced by aerobic microorganisms, and yeasts play a key role in this production (Montes, Hafner, Rotz, & Mitloehner, 2010). The concentration of ethanol found in the 11CFT treatment was 0.70 g kg^{-1}, however it did not differ from the 11C33 treatment (1.61 g kg^{-1}). In review, Zopollatto et al. (2009) verified that the use of lactic acid-producing bacteria in silages of various forages stimulated the production of ethanol, and suggest that these results are due to less control of yeasts.

The control treatment obtained the lowest concentration of propionic acid (0.22 g kg^{-1}) compared to treatments 11CFT (1.28 g kg^{-1}) and 11C33 (2.08 g kg^{-1}), given that the production of lactic acid was lower in this silage, and according to Moon (1983), bacteria producing propionic acid consume lactic acid to produce propionate, acetate and carbon dioxide. It is noteworthy that propionic acid is an important antifungal, which can improve the aerobic stability of the silage.

The control silage, in turn, showed the lowest concentration of butyric acid compared to both inoculated silages. According to Sá, Nussio, Zopollatto, Junges and Bispo (2013), high concentrations of butyric acid denote that there was an action of enterobacteria during the fermentation process, and a greater availability of nutrients present in the inoculated silages may have facilitated the development of these spoilage microorganisms.

Table 3 lists the average data of dry matter, mineral matter and pH during the period of 105 days of use of silages for animal feed. The dry matter contents remained stable both between silages and in different periods of silo unloading. The contents of mineral matter, regardless of the evaluation period, also showed no differences.

Table 3
Average contents of dry matter, mineral matter and pH during 105 days in feed out period

Evalueted periods	Silage			Average
	Control	11CFT	11C33	
		Dry matter, % in NM		
0 to 28 days	42.13	40.75	41.50	41.46
29 to 56 days	38.91	39.84	40.60	39.79
57 to 84 days	40.29	39.10	40.84	40.08
85 to 105 days	42.37	41.07	38.02	40.47
Average	40.81	40.19	40.24	
		Mineral matter, % in DM		
0 to 28 days	3.06	3.19	2.62	2.96 B
29 to 56 days	3.15	3.16	2.70	3.00 B
57 to 84 days	2.35	2.32	2.77	2.48 B
85 to 105 days	3.75	4.68	4.54	4.32 A
Average	3.08	3.34	3.16	
		pH, index		
0 to 28 days	3.95	3.98	4.13	4.02 B
29 to 56 days	4.00	3.90	4.02	3.97 B
57 to 84 days	4.05	3.97	4.85	4.29 A
85 to 105 days	4.54	4.35	4.45	4.44 A
Average	4.13 b	4.05 b	4.36 a	

Averages, followed by different letters in the same row, differ by the Tukey Test at 5%.

Regarding the evaluated periods, the dry matter and the temperature of the silage exposed to air were not affected. The mineral content of the silage was higher (P> 0.05) in the last evaluated periods, which may be related to the longer time of exposure to the environment and greater consumption of soluble nutrients by the microorganisms and the acids themselves, which result in consumption and hydrolysis of carbohydrates and proteins, increasing the content of mineral matter.

Based on the data from the present study, the authors corroborate that, even with the use of inoculants, priority should be given to adequate handling of the silo face during unloading (Li & Nishino, 2011). Rodrigues et al. (2015) also observed an increase in pH values in more advanced periods of silage use, given that the longer time of exposure to yeasts can contribute to greater consumption of lactic acid.

Table 4 lists the summary of the analysis of variance of data relating to temperature, pH, dry matter and mineral content of corn silage treated with different inoculants, at different evaluated periods.

Table 4
Summary of analysis of variance for temperature, pH, dry matter and mineral matter contents of corn silage treated with different inoculants, at different feed out times

Source of mariation	Mean error square			CV, %	Average	Probability	
	Inoculant (I)	Block (B)	Error			I	B
40th day:							
Temperature	225.3137	16.2650	2.0430	5.56	25.71	0.0001	0.0006
pH	13.3286	0.1752	0.1476	6.50	5.91	0.0001	0.3090
Dry matter	0.0074	0.0011	0.0004	4.71	44.62	0.0001	0.0926
Mineral matter	0.5465	0.0045	0.2115	19.00	2.42	0.0797	0.9790
83th day:							
Temperature	226.7946	2.5900	2.1930	6.10	24.27	0.0001	0.3115
pH	14.0102	1.2675	0.2119	10.15	4.53	0.0001	0.0036
Dry matter	0.0030	0.0007	0.0004	3.88	49.26	0.0005	0.1319
Mineral matter	0.4826	0.0629	0.3823	24.15	2.55	0.2878	0.8484

The regression equations in Figure 1 show a quadratic trend for all silages in all evaluated periods. At 83 days (b), the silage inoculated with 11C33 showed the lowest temperatures at the end of the aerobic stability tests. At both times, silage with 11C33 showed a minimum point, while silage inoculated with 11CFT showed curves with a maximum point in both periods. It is explicit in the trend line at 83 days (a) the influence of the ambient temperature on the silage, given that the silage only reduced its temperature during the stability test.

Trends very close to those described for silage with 11CFT were observed in the control silage in all the evaluation periods, showing that the presence of a genus of lactic acid-producing bacteria (*L. plantarum*) can be the great differential brought by the 11C33 inoculant.

Data in figure 2 show that the regression equations for pH, in the two different evaluated periods, had an increasing linear behavior with advancing hours of aerobic exposure. According to Rabelo et al. (2012), the increase in pH occurs due to the consumption of lactic acid by yeasts and also by the breakdown of proteins into ammonia nitrogen by fungi in a second moment. This breakdown of more complex structures, such as proteins, is described just after the beginning of the pH increase, and are responsible for the greatest nutritional losses in the food (Kleinschmit & Kung, 2006).

As observed in the temperature behavior, silage with 11C33 inoculant also showed good control of pH increase at 83 days, while at 40 days the increase was prominent. As highlighted in the past, this silage presented greater differences in relation to the others, while the silage with 11CFT inoculant and control showed similar trends in all periods.

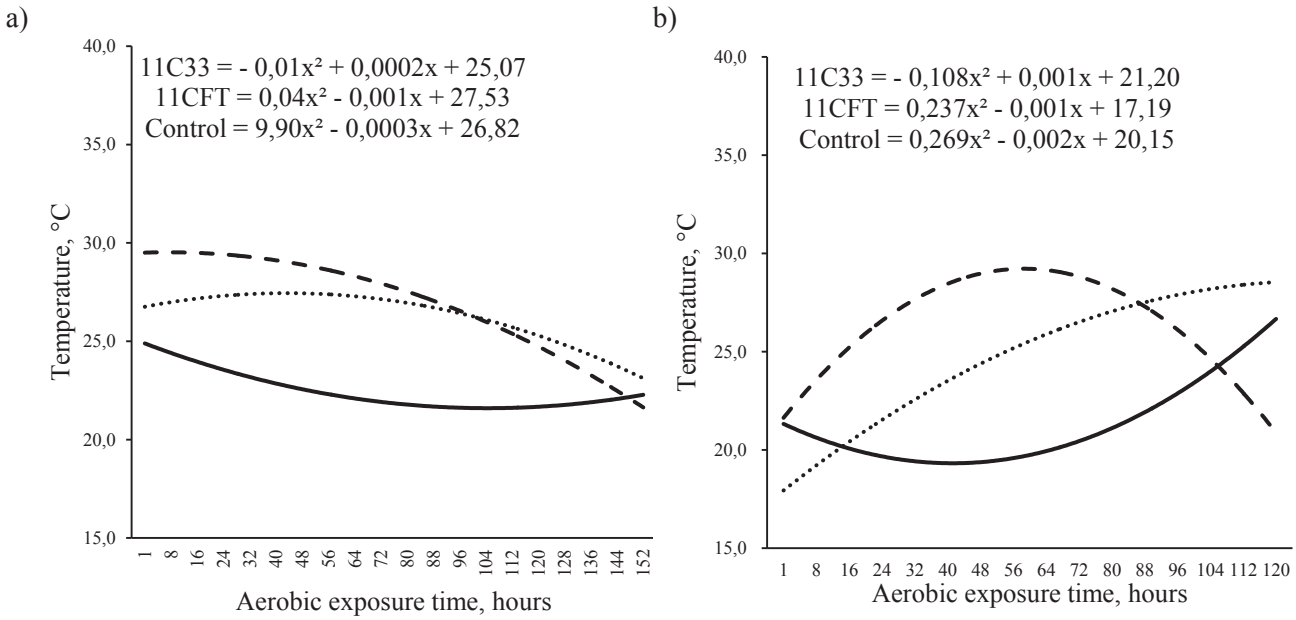

Figure 1. Temperature of corn silage treated with inoculants 11C33 (continuous line), 11CFT (dotted line), and control (dashed line), at 40th day (a) and 83th day (b) after opening the silo.

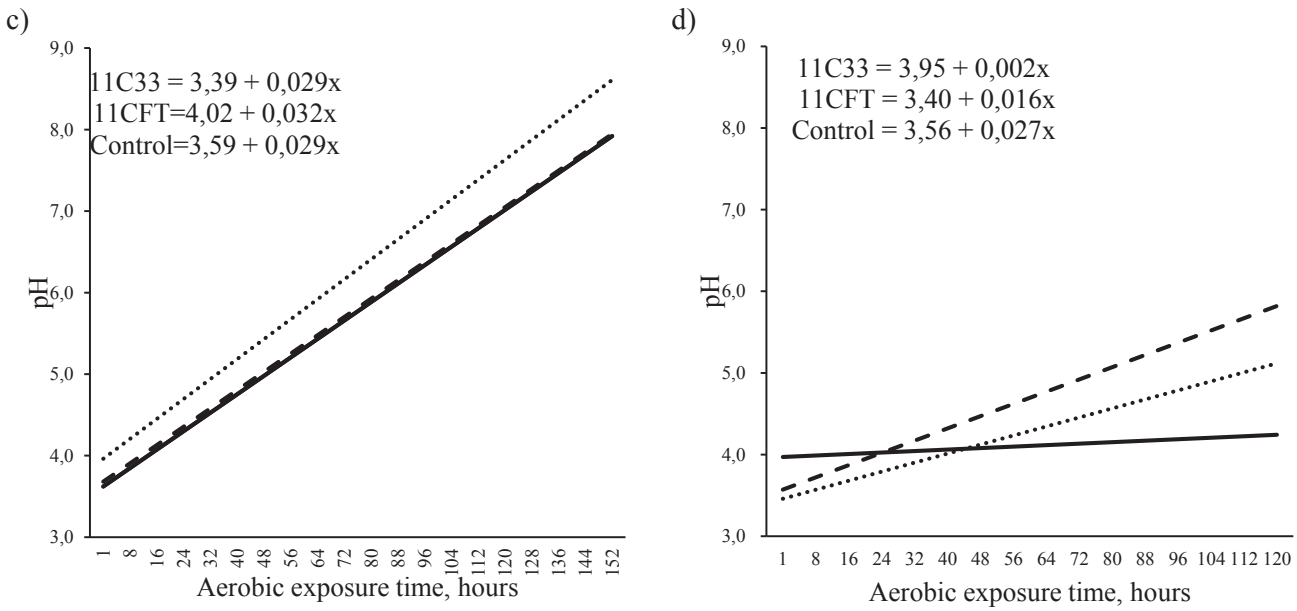

Figure 2. Values of pH of corn silage treated with inoculants 11C33 (continuous line), 11CFT (dotted line), and control (dashed line), at 40th day (c) and 83th day (d) after opening the silo.

The largest increases in pH by time elapsed were observed for silage with 11CFT at 40 days (0.032 pH points hour^{-1}, f), for control silage at 83 days (0.027 pH points hour^{-1}, g). The pH observed after the aerobic stability tests did not maintain the same trends in the different evaluation period (Table 5). There was no difference between the final pH of the silages used at 40 days of use. At 83 days, it is possible to notice that the silage with 11C33 inoculant showed the lowest pH in relation to the silage with 11CFT and control (4.3 against 6.1 and 6.2, respectively).

Coincidentally, these data had relationships with the hours for loss of aerobic stability, where there was no difference in the first evaluation, but, in the second, the silage with 11CFT inoculant and control silage lost their stability with 96.0 and 80.0 hours, respectively, while the silage inoculated with 11C33 did not destabilize during the evaluation time. Therefore, the 11C33 inoculant proved to be more effective for aerobic stability.

Table 5
Aerobic stability of corn silages treated with different inoculants, at different feed out times (40th and 83th day)

	Silage			*P*-value
	Control	11CFT	11C33	
40th day:				
Dry matter, %NM	38.76 b	38.65 b	39.60 a	0.0001
Mineral matter, % DM	2.28	2.42	2.41	0.0797
Stability after pH test	7.7	8.2	7.6	0.5540
Time to maximum temperature, hours	48.3 b	80.0 a	32.0 b	0.0001
Aerobic stability, hours	40.0	40.0	48.0	0.2935
83th day:				
Dry matter, % NM	47.31 a	45.90 b	40.09 c	0.0005
Mineral matter, % MS	2.45 a	2.45 a	2.48 a	0.2878
Stability after pH test	6.2 a	6.1 a	4.3 b	0.0226
Time to maximum temperature, hours	48.0 c	80.0 b	112.0 a	0.0001
Aerobic stability, hours	80.0	96.0	ND	-

Averages, followed by different letters in the same row,differ by the Tukey Test at 5%.
ND: Loss of aerobic stability was not diagnosed during evaluation time.

A. S. Oliveira et al. (2017) evaluated aerobic stability in conventional silage (40.09% dry matter) and inoculated (40.88% dry matter) with *Lactobacillus plantarum* and *Pediococcus acidilactici* at a concentration of 1.0 × 105 CFU g^{-1}, and obtained less aerobic stability in the inoculated wheat silage. Oney et al. (2018) when evaluating moisture difference in corn silage and *Lactobacillus buchneri* as inoculant found that the combination of the inoculant with the low silage moisture positively influenced aerobic stability. In the present study, the

forage moisture content was also high, and only the 11C33 inoculant was superior; the other 11CFT inoculant may not have responded to stability due to the moisture content.

Table 5 also lists that there was no difference between the mineral content of silages, regardless of the evaluation period. In theory, when there is consumption of organic compounds, there is an increase in mineral matter in the silage (Valeriano et al., 2009; Zanette et al., 2012), and this behavior without difference implies that the use of inoculants did not interfere with the consumption of these organic compounds by spoilage microorganisms.

Conclusions

Both inoculants improved the *in situ* digestibility of corn silage compared to control silage. They provide an increase in the content of lactic and propionic acids, which assist to reduce dry matter losses and ethanol production.

There were no variations in temperature and pH at the silo unloading, however, the use of the 11C33 inoculant generated a higher concentration of acetic and propionic acids providing better aerobic stability after exposure to air.

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